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THE ELECTRON MICROSCOPIC AUTORADIOGRAPHY OF TISSUE CULTURE CELLS

INFECTED WITH FOWL PLAGUE VIRUS

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by

G. A. Klisenko, V. M. Stakhanova,
E. M. Zhantiyeva, and V. M. Zhdanov

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G. A. Klisenko, V. M. Stakhanova, E. M. Zhantiyeva, V. M. Zhdanov

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13. ABSTRACT

The feasibility of utilization of radioactive carbon for electron microscopic autography of tissue cultures during maximal infection has been demonstrated.

During the early stages of the development of infection, the maximal quantity of autographs is observed within the nucleus and nucleolus of the infected cell.

During the last stage a sharp increase of the quantity of autographs and their distribution in the nucleus and cytoplasm has been noted.

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THE ELECTRON MICROSCOPIC AUTORADIOGRAPHY OF TISSUE CULTURE CELLS INFECTED WITH FOWL PLAGUE VIRUS

During the last decade the autoradiographic method has found wide application in biological investigations. Nevertheless, it began to be used in conjunction with electron microscopy only comparatively recently. The pioneers in this field were Liquier-Milward [6], who studied a number of tumor cells, labeled with Co^{60}, and O'Brien and George [7] who used Po^{210} during the investigation of ultrathin sections of yeast cells. Later a number of authors devoted their works to different aspects of application of the above-mentioned method. Data on the study of the E. coli [10], ultra t' 'n sections of normal animal tissues [2-4], and the study of tissue cultures [9] by the method of electron microscopic autoradiography have appeared in print. Interesting results were obtained by Hay and Revel, who investigated the localization of intracellular synthesis of DNA [5]. The majority of the authors have used tritium-labeled thymidine in their experiments.

The object of the present work was the utilization of electron microscopy in conjunction with autoradiography for the study of virus infection in tissue culture cells on a microvirus model.

Materials and Methods

The fowl plague virus (FPV) (Waybridge strains of 151st embryonal passage, which passed five serial passages on limited cultures of human embryo in cutaneo-muscular tissue) was used. The titre of the virus was 10^6 TCD_{50}/ml, the hemagglutination titre with 1% suspension of hen erythrocytes - 1:128.

The primary monolayer cultures of the cutaneo-muscular tissue of human embryo were grown on glass slides (glass slips) 2 x 2 cm in size, placed into Petri dishes. Thirty ml of cell suspension, containing 400,000 cells/ml of synthetic medium No. 199 with 10% of bovine serum was placed into each dish containing 5-6 glass slides. On the 4th-5th day the cultures were infected by the fowl plague virus in a dose of 10 ID_{50} per cell. The incubation of the infected cultures was conducted in the same medium with the addition of 0.4 μ Cu adenine of C^{14} per ml.

The infected cultures were investigated 30 min. after and 3 hours after the introduction of the virus. After washing with acetate-veronal buffer (pH 7.5), the cells were fixed for 40 min. with 1% solution of osmium tetraoxide and buffered according to Palade [8] at a temperature of 4° and pH = 7.5. Then followed the usual processing used in the electron microscopic investigations: dehydration in alcohols of increasing concentrations, passing through a mixture of the absolute alcohol and butylmethacrylate (1:1) for 35 min. and of butylmethacrylate without benzoyl peroxide for 40 min. The covering of the cultures was done directly on the glass slides with the help of metal rings [1] by introduction into the prepolymerized mixture of butylmethacrylate with methylmethacrylate in the ratio of 2:1 with addition of benzoyl peroxide. The polymerization was carried out at a temperature of 60° for a period of 24 hours.

Sections 270-300 Å thick were prepared on the LKB Productor ultratome, mounted to grids covered by Formvar film and stained for 20 min. with 1% aqueous solution of uranyl-acetate.

In order to obtain the autographs the sections were covered with the help of a hair loop with a thin layer of fine grained nuclear M emulsion made by Soviet industry and diluted with distilled water 1:5. After a 5-day exposure at room temperature the emulsion was developed in an amidol developer for 5 min., fixed by hyposulfite, and th . washed with distilled water. The dried sections were examined under the UEM-5-U electron microscope.

Sections of the noninfected cultures of cutaneo-muscular tissue of human embryo, grown in a medium without the addition of isotope, and sections of the same culture incubated in the presence of the isotope, both covered by emulsion, were used as the controls.

Results

Figure 1 shows sections of normal, noninfected cells of cutaneo-muscular tissue from human embryos. The upper portion of the photograph is occupied by the nucleus with the nucleolus; the lower, by the cytoplasm, in which mitochondria can be clearly seen.

Figure 2 shows a characteristic "star," consisting of autographs of various types, obtained upon fission of C^{14} -adenine outside of the tissue. In the middle of the autographs, structures in the form of balls of twisted threads may be distinguished, and dotted autographs in the form of solitary granules or aggregates of them.

During electron microscopy of emulsion-coated ultrathin sections of the control, noninfected culture, incubated in the presence of isotope, uniform distribution of single autographs, representing irradiated granules of emulsion at the site of discharge of β-particles upon disintegration of the C^{14}, was noted in the nucleus and cytoplasm of the cells.

In micrographs of sections of infected cultures, incubated in medium containing isotope, it is clear that the character of the distribution of autographs is different from that in the controls. Thirty minutes after infection most of the autographs are concentrated in the region of the nucleolus, in the nucleus, and at the boundary of nucleus and cytoplasm (Figure 3). Three hours after addition of virus, the entire cell (both nucleus and cytoplasm) is uniformly covered with autographs, to the extent that subcellular structures are almost indistinguishable (FIgure 4).

Discussion

The investigations have shown the feasibility of the utilization of electron microscopic autography method for the study of virus infection in a tissue culture. The β-emitter C^{14} was used in the compound adenine, a precursor of RNA. The choice of the adenine was determined by the fact that it penetrates a cell easily, the speed of its incorporation is smaller than the speed of destruction and it enters into only one type of macro-molecule -- into the nucleic acid. Since the virus of the true fowl plague contains only RNA, the intensive incorporation of the C^{14} -adenine into the cells of the infected culture observed by us is connected with the change of metabolism of the RNA during the virus infection.

The intensive incorporation of the labeled precursor into the nucleus and especially into the nucleolus during the early stage of the infection development testifies to the leading role of these cell components during

the initial phase of the latent period of the virus reproduction. It seems that the virus during the first minutes after its penetration into the cells is accumulated mainly in the nucleolus and starts intense synthesis of the RNA there.

The presence of autographs of 2 types, observed on the microphotographs can probably be explained by the presence of different energy particles in the spectrum of C^{14} β-fission. For example, Choi obtained point autographs [3] using the S^{35} -labeled compounds for the electron microscpic autography; on the other hand a number of authors working with the H^3, which is a weak emitter, show autographs in the form of twisted threads [2, 4, 5, 9].

The maximal quantity of the point (dot) autographs analogous to the S^{35} autographs mentioned by us is explained, it seems by the closeness of the energies of the β-particles of these isotopes. Nevertheless, it is possible that the regimen of the preparation compounds plays a certain role here.

Conclusions

1. The feasibility of utilization of radioactive carbon for electron microscopic autography of tissue cultures during maximal infection has been demonstrated.

2. During the early stages of the development of infection, the maximal quantity of autographs is observed within the nucleus and nucleolus of the infected cell.

3. During the last stage a sharp increase of the quantity of autographs and their distribution in the nucleus and cytoplasm has been noted.

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GRAPHICS NOT REPRODUCIBLE

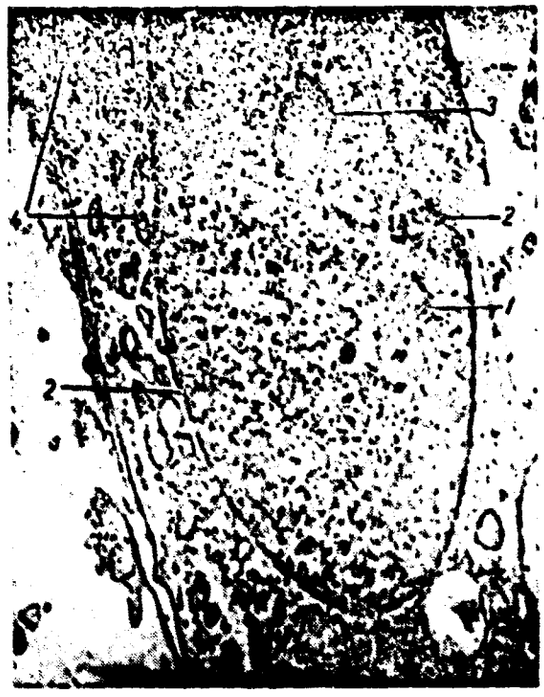

Figure 1. Electron micrograph of a section of uninfected cell of cutaneo-muscular tissue of human embryo.
1 - nucleus, 2 - nuclear membrane; 3 - nucleolus; 4 - mitochondrion.
X 12000 magnif.

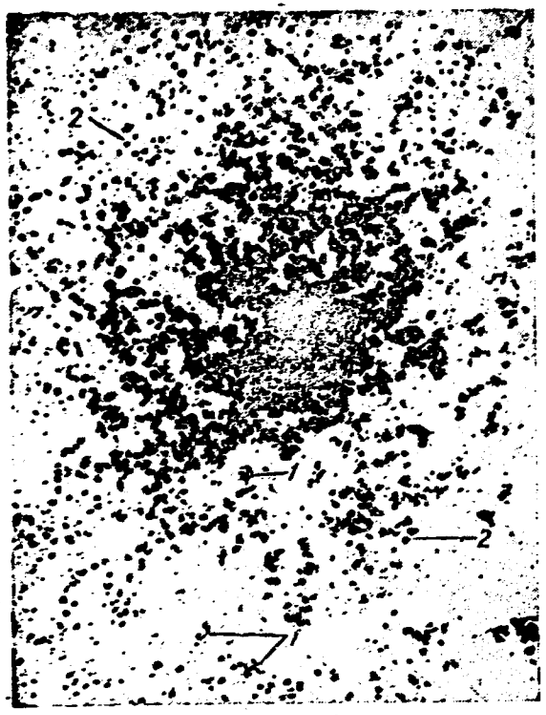

Figure 2. An electron micrograph of a "star" obtained during the fission of the C^{14} -adenine outside of tissue.
1- autographs in the form of twisted threads;
2- autographs in the form of granules.
3- autographs x 15000 magnif.

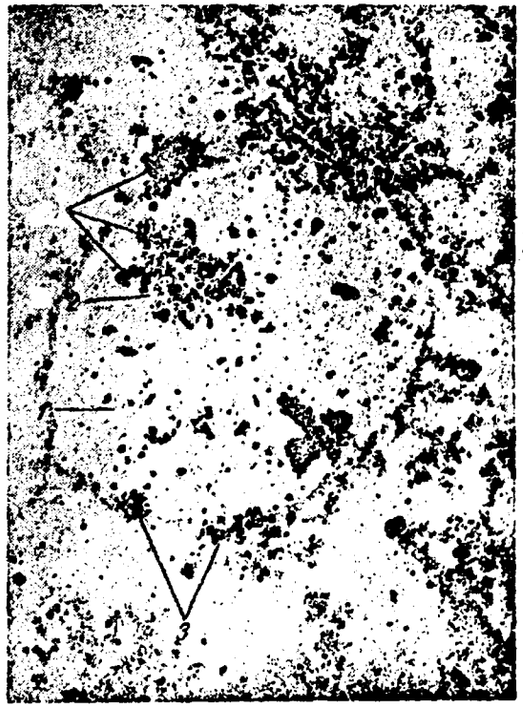

Figure 3. An electron microscopic micrograph of a section of the cutaneo-muscular tissue of human embryo infected by the FPV virus and incubated in the presence of the C^{14} -adenine 30 min. after infection.
1- nucleus; 2-, nucleolus; 3- autographs x 12000 magnif.